

In the Claims

1. (Currently Amended) A method to evaluate the presence of high levels of autoantibodies against endothelial protein C (PC) / activated PC receptor (EPCR) in a sample, ~~said method characterised by~~ comprising ~~quantifying the in vitro quantification of~~ autoantibodies against EPCR in said sample from a subject.
2. (Currently Amended) Method according to claim 1, ~~characterised by~~ wherein said presence of high levels of autoantibodies against EPCR being related to a pathology selected from the group consisting of autoimmune disease, vascular disease and obstetric complications.
3. (Currently Amended) Method according to ~~any of claim~~ claims 1 or 2, ~~characterised in that~~ wherein said autoimmune disease is selected from the group consisting of antiphospholipid syndrome, systemic lupus erythematosus, rheumatoid arthritis and autoimmune vasculitis.
4. (Currently Amended) Method according to ~~any of claim~~ claims 1 or 2, ~~characterised in that~~ wherein said vascular disease is selected from the group consisting of arterial vascular disease, venous vascular disease and thrombosis of the microcirculation.
5. (Currently Amended) Method according to claim 4, ~~characterised in that~~ wherein said vascular disease is selected from the group consisting of myocardial infarction, cerebral stroke, a transient cerebrovascular accident, limb ischemia, atherosclerosis, aneurysm, thrombosis, superficial venous thrombosis, deep venous thrombosis, and pulmonary embolism.
6. (Currently Amended) Method according to ~~any of claim~~ claims 1 or 2, ~~characterised in that~~ wherein said obstetric complication is selected from the group consisting of miscarriage, fetal death, premature birth, delayed intrauterine growth, eclampsia and pre-eclampsia.
7. (Currently Amended) Method according to ~~any of claims~~ claim 1 to 6, ~~characterised in that~~ wherein ~~said the mentioned sample comprises is a sample of~~ serum or plasma.
8. (Currently Amended) Method according to ~~any of claims~~ claim 1 to 7, ~~characterised in that~~ wherein ~~said the mentioned~~ subject is human.

9. (Currently Amended) Method according to ~~any of claims claim 1 to 8, characterised in that~~ wherein quantification of ~~these anti-EPCR autoantibodies~~ against EPCR is carried out by means of an immunoassay coupled to a marker.

10. (Currently Amended) Method according to ~~any of claims claim 1 to 9, characterised in that~~ wherein quantification of ~~these anti-EPCR autoantibodies~~ against EPCR is determined using ~~carried out by means of~~ an ELISA test, said test comprising:

- a) ~~immobilizing solid support immobilization of an~~ immobilizable polypeptide comprising the EPCR amino acid sequence or a fragment thereof containing at least one epitope that can be recognized by an anti-EPCR autoantibody on a solid support;
- b) ~~incubation of incubating~~ the immobilized polypeptide with the ~~[[a]]~~ sample ~~suspected to contain anti-EPCR autoantibodies~~, obtained from the subject~~[[,]]~~ for sufficient time to allow binding of the anti-EPCR autoantibodies to the immobilized polypeptide, and the formation of polypeptide-anti-EPCR autoantibody complexes;
- c) ~~removal of the removing excess remaining~~ sample not bound to the immobilized polypeptide; and
- d) ~~incubation of incubating~~ the polypeptide-anti-EPCR autoantibody complexes with a second antibody conjugated to an enzyme, where the second antibody is able to bind to the ~~these~~ anti-EPCR autoantibodies.

11. (Currently Amended) Method according to claim 10, ~~characterised in that~~ wherein ~~the mentioned~~ said immobilizable polypeptide is selected from the group consisting of ~~between~~:

- a) a polypeptide comprising the sequence of amino acids of full length EPCR; and
- b) a polypeptide comprising the sequence of amino acids of a fragment of EPCR containing at least one epitope capable of being recognized by an anti-EPCR autoantibody.

12. (Currently Amended) Method according to ~~any of claims 1 to claim 11, characterised in that~~ wherein said immobilizable polypeptide comprises is a fusion protein comprising:

- a) a region A ~~composed of~~ comprising a first polypeptide containing the EPCR amino acid sequence or a fragment thereof containing at least one epitope capable of being recognized by an anti-EPCR autoantibody; and
- b) a region B ~~composed of~~ comprising a second polypeptide comprising a sequence of amino acids of use for isolating or purifying the mentioned fusion protein, and/or a sequence of amino acids of use for anchoring the mentioned fusion protein to a solid support.

13. (Currently Amended) Method according to claim 12, (Currently Amended) said region B is bound to the amino terminal extreme of region A.

14. (Currently Amended) Method according to claim 12, ~~characterised in that~~ wherein said region B is bound to the carboxyl terminal extreme of region A.

15. (Currently Amended) Method according to ~~any of claims claim 12 to 14, characterised in that~~ wherein said region A comprises the amino acid sequence of the soluble part of human EPCR.

16. (Currently Amended) Method according to ~~any of claims claim 12 to 14, in which~~ wherein the amino acid sequence of use for isolating or purifying the mentioned fusion protein, and/or an amino acid sequence of use for anchoring said fusion protein to a solid support present in region B, comprises a sequence ~~of amino acids~~ selected from the group consisting of Arg-tag, His-tag, FLAG-tag, Strep-tag, an epitope capable of being recognized by antibody, SBP-tag, S-tag, calmodulin binding peptide, cellulose binding domain, chitin binding domain, glutathione S-transferase-tag, maltose binding protein, NusA, TrxA, DsbA, Avi-tag, Ala-His-Gly-His-Arg-Pro (SEQ ID NO: 4) (2, 4, and 8 copies), Pro-Ile-His-Asp-His-Asp-His-Pro-His-Leu-Val-Ile-His-Ser (SEQ ID NO: 5), Gly-Met-Thr-Cys-X-X-Cys (SEQ ID NO: 6) (6 repetitions), \square -galactosidase and VSV-glycoprotein.

17. (Currently Amended) Method according to ~~any of claims claim 12 to 16, characterised in that~~ wherein region B comprises ~~is composed of~~ a polypeptide comprising a c-myc epitope capable of being recognized by an anti-c-myc antibody and a tail of histidines (His-tag).

18. (Currently Amended) Method according to ~~any of claims claim 12 to 17, characterised in that~~ wherein said immobilizable polypeptide is a fusion protein comprising the sequence of amino acids of the soluble part of human EPCR, the sequence of amino acids corresponding to c-myc epitope and a tail of histidines (His-tag).

19. (Currently Amended) Method according to ~~any of claims claim 12 to 18, characterised in that~~ wherein said immobilizable polypeptide is a fusion protein ~~whose sequence of amino acids is shown in~~ comprising SEQ ID NO: 3.

20. (Currently Amended) Method according to claim 10, ~~characterised in that~~ wherein said second antibody is an immunoglobulin isotype-specific antibody originating from a species different to that of the subject whose sample is being tested.

21. (Currently Amended) Method according to ~~claims 10 or~~ claim 20, ~~characterised in that wherein~~ said ~~second~~ immunoglobulin isotype-specific antibody is selected from the group consisting of an anti-human IgG antibody, an anti-human IgM antibody, an anti-human IgA antibody, and their mixtures.

22. (Currently Amended) Method according to ~~any of claims~~ claim 20 or 21, ~~characterised in that wherein~~ said second antibody is conjugated to an enzyme selected from ~~between~~ peroxidase or and alkaline phosphatase.

23. (Currently Amended) Method according to ~~any of claims~~ claim 1 to 22, ~~characterised in that it moreover comprises~~ further comprising the comparison of comparing quantified anti-EPCR autoantibody levels ~~determined in the sample from the subject versus~~ to normal levels of anti-EPCR autoantibody levels.

24. (Currently Amended) A method according to claim 1, ~~characterized in determining wherein~~ the variation in the levels of anti-EPCR autoantibodies are quantified over a given time period.

25. (Currently Amended) Method according to claim 24, ~~characterised in that wherein~~ said sample originates from a subject previously diagnosed with an autoimmune or vascular disease, or who has suffered an obstetric complication, and is subject to therapeutic treatment.

26.-33. (Cancelled)

34. (Currently Amended) A kit for *in vitro* evaluation of the presence of high levels of autoantibodies against EPCR in a sample, ~~characterised in that~~ said kit ~~comprises~~ comprising an immobilizable polypeptide that comprises the EPCR amino acid sequence or a fragment thereof containing at least one epitope capable of being recognized by an anti-EPCR autoantibody.

35. (Currently Amended) ~~[[A]]~~ The kit according to claim 34, ~~characterised in that wherein~~ said immobilizable polypeptide comprises is a fusion protein comprising:

- i) a region A ~~composed of~~ comprising a first polypeptide containing the EPCR amino acid sequence or a fragment thereof containing at least one epitope capable of being recognized by an anti-EPCR autoantibody; and
- ii) a region B ~~composed of~~ comprising a second polypeptide comprising an amino acid sequence of use for isolating or purifying the mentioned fusion protein, and/or an amino acid sequence of use for anchoring the mentioned fusion protein to a solid support.

36. (Currently Amended) [[A]] The kit according to claim 35, ~~in that~~ wherein said region A is ~~characterized by comprising~~ comprises the amino acid sequence of the soluble part of human EPCR.

37. (Currently Amended) [[A]] The kit according to ~~any of claims~~ claim 35 or 36, ~~characterised in that~~ wherein said immobilizable polypeptide is a fusion protein comprising the amino acid sequence of the soluble part of human EPCR, the amino acid sequence corresponding to c-myc epitope and a tail of histidines (His-tag).

38. (Currently Amended) [[A]] The kit according to ~~any of claims~~ claim 35 to 37, ~~characterised in that~~ wherein said immobilizable polypeptide is a fusion protein ~~with the sequence of amino acids shown in~~ comprising SEQ ID NO: 3.